

Please insert the attached Sequence Listing as new pages --48-66--.

**IN THE CLAIMS**

Please renumber the Claims pages from pages "48-54" to --67-85--.


**REMARKS**

The specification has been amended to provide sequence identifiers. Applicants' amendments do not introduce new matter.

The Examiner has requested that a Sequence Listing be provided. Applicants submit this Preliminary Amendment and Response to provide as a separate part of the disclosure, a "Sequence Listing" pursuant to 37 C.F.R. §§ 1.821-1.825. Applicants submit herewith in paper copy and on floppy disk the Sequence Listing in computer readable form. The contents of the paper and computer readable copies are the same and include no new matter.

Applicants respectfully request entry of this Preliminary Amendment prior to examination of the present application.

Dated: June 21, 2001

  
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**APPENDIX I**  
**MARKED-UP VERSION OF SPECIFICATION'S REPLACEMENT PARAGRAPHS**

The following is a marked-up version of the specification's replacement paragraphs pursuant to 37 C.F.R. §1.121(b).

**IN THE SPECIFICATION**

On page 11, please delete the paragraph beginning on line 19 and ending on line 31, and replace with the following paragraph:

Figures 1A (SEQ ID NO: 1) and 1B (SEQ ID NOS: 4-9) show the sequence analysis of p60 katanin. Figure 1A: Predicted protein sequence of the *S. purpuratus* katanin p60 subunit (GENBANK AF052191). Sequences obtained by direct peptide microsequencing are underlined. Differences between the predicted peptide sequence and that obtained by direct sequencing are indicated by double underlines (S95 was reported as F, H99 was reported as P, and P138 was reported as T). The Walker A (P-loop) motif is shaded. Figure 1B: Amino acid sequence alignment of the p60 AAA domain with AAA members mei-1 (*C. elegans*, GenBank L25423), Sug1p (*S. cerevisiae*, GenBank X66400), ftsH (*E. coli*, GenBank M83138), Pas1p (*S. cerevisiae*, GenBank M58676), and NSF (*C. longicaudatus*, GenBank X15652). Identical residues are shaded black, residues conserved in >60% of the shown members are shaded gray. Left hand numbering indicates the amino acid residue in the corresponding sequence. Alignment was performed using PILEUP (Genetics Computer Group) and the output was shaded using MACBOXSHADE.

On page 11, please delete the paragraph beginning on line 32 and ending on page 12, line 10, and replace with the following paragraph:

Figures 2A (SEQ ID NO: 2) and 2B (SEQ ID NOS: 10-13) show the sequence analysis of p80 katanin. Figure 2A: Predicted protein sequence of the *S. purpuratus* katanin p80 subunit (GENBANK AF052433). Sequences obtained by direct peptide microsequencing are underlined. Differences between the predicted peptide sequence and that obtained by direct peptide sequencing, or differences found between 2 different p80 cDNA clones are

indicated by double underlines. Figure 2B: Amino acid sequence alignment of the WD40 repeat region of p80 with a putative human ortholog of p80 (Hs p80, GenBank AF052432), TFIID (*Homo sapiens*, GenBank U80191), and putative serine/threonine kinase PkwA (*Thermomonospora curvata*, GenBank P49695). Identical residues are shaded black, residues found in at least 2 sequences are shaded in grey. Left hand numbering indicates the amino acid residue in the corresponding sequence. Alignment was performed using PILEUP (Genetics Computer Group) and the output was shaded using MACBOXSHADE.

On page 36, please delete the paragraph beginning on line 2 and ending on line 15, and replace with the following paragraph:

Deciphering the roles of the two katanin subunits is essential for understanding the enzyme's mechanism and biological activities. However, separation of the native sea urchin p60/p80 subunits requires denaturing conditions. We therefore sought to express the two subunits together and separately and then test their enzymatic activities. Bacterial expression of p60 produced largely insoluble protein, and the small amount of soluble p60 had no microtubule-stimulated ATPase activity (data not shown). However, using the baculovirus expression system, we obtained soluble p60, p80, and the p60/p80 complex (each expressed with a N-terminal His<sub>(6)</sub> tag) (SEQ ID NO: 14), and purified the expressed proteins using metal affinity chromatography Fig. 3A). When p60 and p80 were co-expressed, the stoichiometry of the two subunits in the purified protein was approximately equal (1.0:0.9 p60:p80 molar ratio, as determined by Coomassie staining). Moreover, immunoprecipitation with an anti-p60 antibody led to co-immunoprecipitation of equal quantities of p60 and p80 (Fig. 3B). These results indicate that baculovirus-expressed p60 and p80 heterodimerize, as observed with native katanin (McNally and Vale (1993) *Cell*, 75:419-429).

On page 42, please delete the paragraph beginning on line 25 and ending on line 31, and replace with the following paragraph:

Katanin was purified from extracts of *S. purpuratus* eggs essentially as described previous (McNally and Vale (1993) *Cell*, 75:419-429), except that the hydroxyapatite chromatography was carried out using a Pharmacia HR10/30 column packed with 20 µm ceramic hydroxyapatite beads (American International Chemical, Natick, MA). Internal

peptide sequences of the p60 and p80 subunits were obtained from native sea urchin katanin as described (Iwamatsu (1992) *Electrophoresis* 13: 142-147). Two additional p80 peptides were also obtained: DASMMAM (SEQ ID NO: 15) and IQGLR (SEQ ID NO: 16).

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